

# 2013 *Vibrio parahaemolyticus* Season Review



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## Illness Summary:

In 2013 there were a total of 109 *Vibrio parahaemolyticus*-associated laboratory confirmed or epidemiologically-associated illnesses potentially implicating Washington molluscan shellfish. Of those 109 illnesses, 30 were not attributed to commercial growing areas for the following reasons:

- 9 illnesses were due to recreational harvest of oysters.
- 4 illnesses were unable to be traced back due to the inability to determine a source in the illness investigation. The individuals consumed raw oysters at parties or purchased them from vendors at fairs and other events.
- 17 illnesses were traced to commercially harvested product, but the illness investigation revealed post-harvest abuse issues either in the transport and handling of product or at the retail level. In addition to multiple sporadic illnesses, this category also includes one illness outbreak where product was abused in transit.

The remaining 79 illnesses were attributed to commercial growing areas in Washington. Among these illnesses, about half occurred in out of state residents who either visited Washington and consumed oysters in the state or consumed oysters at an out of state establishment. The majority of these individuals were from British Columbia, but Washington responded to illnesses from residents of Arizona, California, Colorado, Connecticut, Illinois, Michigan, New Jersey, Ohio, Oregon, Texas, Virginia, and Wisconsin.

Of the commercial illnesses attributed to Washington growing areas:

- 24 were single-source illnesses with complete traceback to one growing area in Washington.
- 55 were multi-source illnesses with incomplete traceback, either due to the consumption of oysters from multiple areas or the inability to determine the precise source of the oysters in the illness investigation. Of these illnesses:
  - o 16 were traced back to only growing areas in Washington.
  - o 27 were traced back to growing areas in Washington and Canada.
  - o 6 were traced back to growing areas in Washington and other states.
  - o 6 were traced back to growing areas in Washington, other states and Canada.

Since there were 24 single-source illnesses with traceback to a Washington growing area and 16 multi-source illnesses with only Washington product implicated, the total number of *Vibrio parahaemolyticus*-associated laboratory confirmed or epidemiologically-associated illnesses from commercially harvested product in Washington with no known post-harvest abuse with product traceback is 40.

## Regulatory Actions Taken:

All 79 single- and multi-source illnesses were used in determining when to initiate a time of harvest to temperature control reduction or growing area closure under Washington's *Vibrio parahaemolyticus* Control Plan (WAC 246-282-006).

In the 2013 *Vp* season Washington instituted time of harvest to temperature control reductions in 18 growing areas due to the occurrence of two sporadic illnesses with harvest dates within a 30 day period. All of these reductions remained in effect until either the growing area met the requirements for closure (two additional illnesses within a 30 day period) or until the end of the control months (September 1 for coastal growing areas, October 1 for inland growing areas). The only exception was the case of Port Gamble Bay growing area. The area underwent a time reduction on 7/22/13, but upon completion of the illness investigation it was determined that there was post-harvest abuse of the product and the illnesses were not attributed to the growing area. The reduction was lifted on 7/29/13.

The time reduction for Peale Passage on 8/8/13 was lifted on 8/19/13 due to the determination by the Public Health Lab (PHL) that the infective agent was *E. coli*. The time reduction was reinstated on 8/22/13 when the PHL determined the individual was infected with *E. coli* and *V. parahaemolyticus*.

Below is a summary of the time of harvest to temperature control reductions:

Growing Area	Reduction date	Reduced from/to (hrs.)	Remarks
Oakland Bay	7/17/2013	4 / 3	2 sporadic illnesses in 30 days
Totten Inlet	7/17/2013	4 / 3	2 sporadic illnesses in 30 days
Bay Center	7/17/2013	10 / 9	2 sporadic illnesses in 30 days
Port Gamble	7/22/2013	4 / 3	2 sporadic illnesses in 30 days, lifted 7/29/13 (illnesses not attributed to growing area)
Hammersley Inlet	8/1/2013	4 / 3	2 sporadic illnesses in 30 days
Dabob Bay	8/5/2013	4 / 3	2 sporadic illnesses in 30 days
Grays Harbor	8/8/2013	10 / 9	2 sporadic illnesses in 30 days
Pickering Passage	8/8/2013	4 / 3	2 sporadic illnesses in 30 days
Reach Island	8/8/2013	4 / 3	2 sporadic illnesses in 30 days
Samish Bay	8/8/2013	4 / 3	2 sporadic illnesses in 30 days
Peale Passage	8/8/2013	4 / 3	2 sporadic illnesses in 30 days, revoked 8/19, re-instated 8/22
Burley Lagoon	8/20/2013	4 / 3	2 sporadic illnesses in 30 days
Eld Inlet	8/20/2013	4 / 3	2 sporadic illnesses in 30 days
Hood Canal 3	8/27/2013	4 / 3	2 sporadic illnesses in 30 days
Hood Canal 1	9/13/2013	4 / 3	2 sporadic illnesses in 30 days
Hood Canal 6	9/13/2013	4 / 3	2 sporadic illnesses in 30 days
Skookum Inlet	9/24/2013	4 / 3	2 sporadic illnesses in 30 days
Port Townsend	9/27/2013	4 / 3	2 sporadic illnesses in 30 days

In the 2013 *Vp* season, Washington instituted growing area closures in 15 growing areas due to the occurrence of four sporadic illnesses with harvest dates within a 30 day period or *t/h* levels greater than 10,000 MPN/g. There were nine closures due to the occurrence of four sporadic illnesses. These closures all occurred among inland growing areas in August and September and lasted through the end of the control months. There were eight closures due to *t/h* levels. These closures occur under the NSSP Section IV Chapter II Protocol .13 and are lifted once the PHL processes two samples collected no less

than seven days apart that are below the action level. These closures occurred in July and August and typically lasted 3-4 weeks.

There were two growing areas that experienced both *tlh* and illness closures. In the case of Hammersley Inlet, the growing area was closed on 8/12/13 due to high *tlh* levels and then remained closed for the rest of the control months due to the occurrence of four sporadic illnesses, which were reported while the area was closed due to *tlh* levels. In the case of Dabob Bay, the growing area was closed due to high *tlh* levels on 8/12/13 and re-opened on 8/26/13. The growing area was then closed on 9/27/13 due to the occurrence of four sporadic illnesses. Although the illnesses occurred in July and early August, a delay in reporting led to the late closure. The Office of Shellfish and Water Protection (OSWP) works closely with Communicable Disease Epidemiology (CD-epi) staff and local health counterparts to ensure the most timely response possible, but reporting delays do still occur especially among out of state illness reports. Below is a summary of the growing area closures:

Growing Area	Closure Date	Reopening Date	Remarks
Hood Canal 8	7/5/2013	8/8/2013	NSSP, <i>tlh</i> >10,000 MPN/gram
Hood Canal 9	7/5/2013	8/5/2013	NSSP, <i>tlh</i> >10,000 MPN/gram
Hood Canal 7	7/10/2013	8/5/2013	NSSP, <i>tlh</i> >10,000 MPN/gram
Quilcene Bay	7/19/2013	8/19/2013	NSSP, <i>tlh</i> >10,000 MPN/gram
Oakland Bay	8/1/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days
Hood Canal 3	8/2/2013	8/27/2013	NSSP, <i>tlh</i> >10,000 MPN/gram
Hood Canal 4	8/2/2013	8/19/2013	NSSP, <i>tlh</i> >10,000 MPN/gram
Totten Inlet	8/8/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days
Hammersley Inlet	8/12/2013	NA	NSSP, <i>tlh</i> >10,000 MPN/gram
Dabob Bay	8/12/2013	8/26/2013	NSSP, <i>tlh</i> >10,000 MPN/gram
Reach Island	8/12/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days
Samish Bay	8/14/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days
Hammersley Inlet	8/15/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days
Pickering Passage	8/15/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days
Burley Lagoon	9/17/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days
Peale Passage	9/19/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days
Dabob Bay	9/27/2013	10/1/2013	WAC, 4 sporadic illnesses in 30 days

### Coordination with FDA:

Throughout the 2013 *Vp* season, OSWP remained in close contact with FDA by providing weekly narrative updates and a copy of the Shellfish Illness Log tracking sheet to the Pacific Coast Director of State Cooperative Programs and the Regional Shellfish Specialists (RSS). There was a great deal of communication with the FDA leading up to, during, and while completing the product recall in response to the one illness outbreak. OSWP worked closely with Center for Food Safety and Applied Nutrition (CFSAN) and the Gulf Coast Seafood Laboratory (GCSL) in conducting *Vp* research. OSWP hosted Dr. Andy DePaola and Dorothy-Jean McCoubrey during their week long field visit to Washington in July while kicking off the GCSL research effort. See research studies portion of report for details on study purpose, methods and results.

## Outreach to Commercial Shellfish Industry:

OSWP sent multiple industry notices out this season. In addition to notices regarding the beginning and ending of the control months, OSWP also:

- Developed and sent a flowchart of the 2011 NSSP time to temperature controls for harvesters and dealers;
- Issued a July reminder of the control plan requirements with recommendations for additional cooling due to an expected heat wave; and
- Developed and sent recommendations for best management practices during *Vp* season in consultation with the *Vp* Advisory Committee.

The best management practices document included recommendations regarding chilling product, information on times, tides, and conditions that are more and less favorable to *Vp* growth, and information regarding *Vp* growth rates at different temperatures.

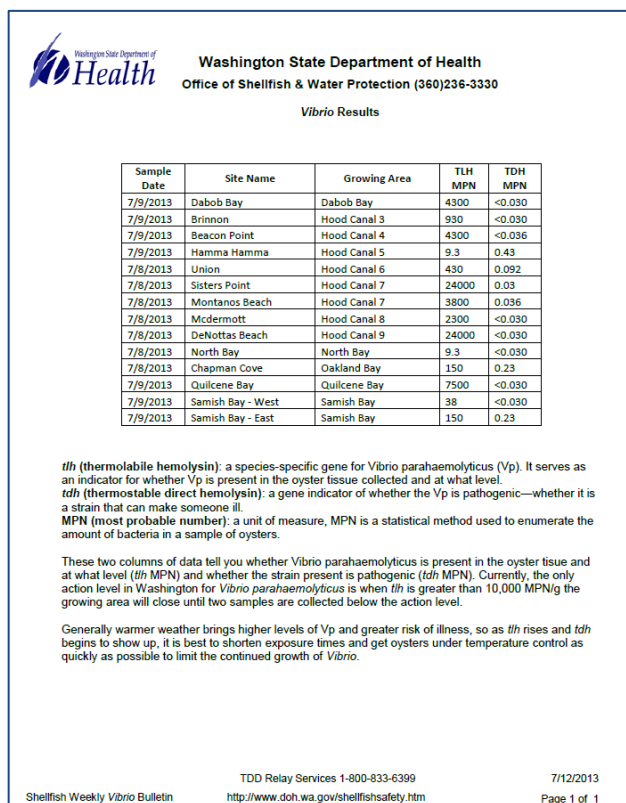


Figure 1. Example of weekly environmental sample results.

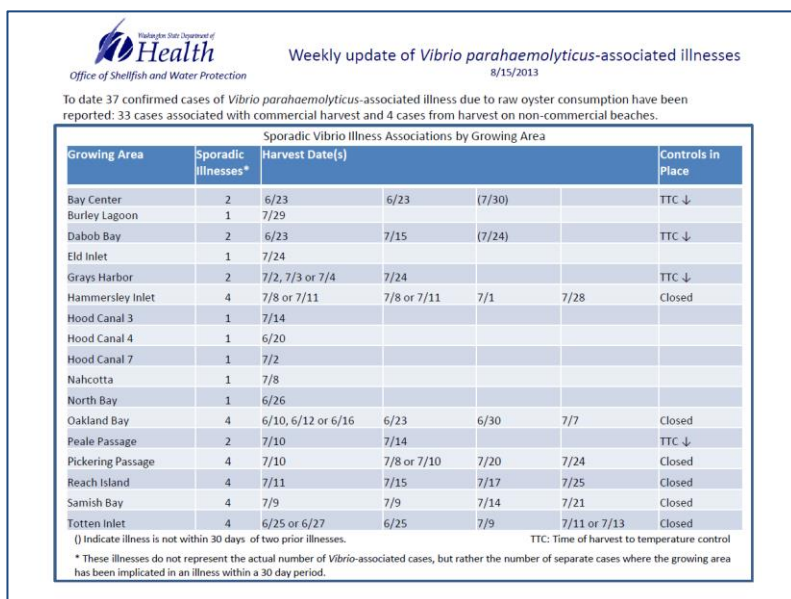


Figure 2. Example of weekly illness report.

track how many illnesses have been attributed to which growing area. OSWP hopes that these resources will encourage harvesters to make good harvesting decisions and provide them the means to

OSWP created new weekly outreach materials to the shellfish industry in 2013. OSWP sent weekly reports of the *tlh* and *tdh* levels collected through *Vp* monitoring efforts (Figure 1) that provided shellfish harvesters the tools to understand whether and how much *Vp* is present in growing areas. In following the advice of the *Vp* Advisory Committee, OSWP also developed a weekly illness report (Figure 2) and began distribution of that report on August 1. This report provided shellfish harvesters the tools to



monitor *Vp* levels and track illnesses as close to real time as possible. Keeping the shellfish industry informed and engaged in *Vp* management fosters good relationships and encourages dialogue.

OSWP also provided more consistent communication to the shellfish industry with regard to *Vp* closures by integrating *Vp* closures into the growing area closure database and growing area closure report. This step helps to ensure that OSWP staff and shellfish harvesters and dealers are all well informed of all growing area closures, regardless of the cause of the closure.

## **Outreach to the Public:**

In addition to targeted communication to the shellfish industry, Washington State Department of Health issued two press releases during the *Vp* season. The first press release, “State warns warm weather raises raw oyster risks”, was issued July 2 in advance of Independence Day. The second news release, “Raw oyster consumptions: state health officials see rise in related illnesses”, was issued August 13 after the occurrence of *Vp* -associated illnesses. This press release coincided with a release by King County on increased *Vp* -associated illnesses. In addition to the press releases, OSWP issued 10 *Vp*-associated messages on the Recreational Shellfish Program’s Facebook page (Figure 3). The messages ranged from information on *Vp* growth rates to advice to cook shellfish in the summer months. In addition to using Facebook to reach out, the Recreational Program coordinator attended multiple festivals, including two ShellFest events organized by Washington State Parks

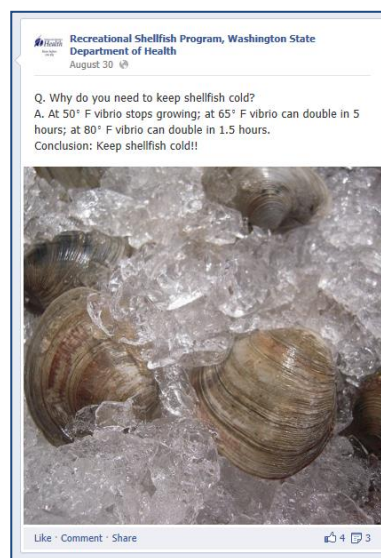


Figure 3. Most popular *Vp*-related Facebook post.



Figure 4. *Vp* advisory sign posted along Hood Canal.

Foundation. OSWP updated the Clickable Maps from June-September with an advisory to cook all shellfish and also posted signs with the help of local counterparts on beaches known to have recreational oyster harvest (Figure 4). The office developed an updated *Vp* Factsheet and new outreach display centered around the message “Check, Chill, Cook!”, which encouraged recreational shellfish harvesters to check the Department of Health’s website before harvesting, chill shellfish from harvest to table, and cook thoroughly in the summer months.

## **Improvements to Internal Tracking:**

OSWP continued to promote the use of a shared email inbox for illness reporting. The shared inbox ensures a timely response to illness reports and improves coordination both internally within OSWP and between OSWP, CD-epi, and local health. OSWP developed an illness tracking tool specific to growing area time-to-temperature reductions and closures beyond the Shellfish Illness Log. This tracker was updated with harvest dates and illness case numbers by growing area as soon as shellfish tags were

received. Maintaining this tracker on the shared drive allowed for quick and efficient evaluation of a growing area's status with regard to regulatory action and provided the basis for the weekly industry updates. In addition, maps were posted to allow all OSWP staff to quickly assess which growing areas had illnesses associated with them, which areas were under a time to temperature reduction, and which areas were closed.

## Environmental Monitoring Efforts:

Health collected 311 environmental samples from June to September. The samples were collected from a total of 31 sites in 24 growing areas. Of those growing areas, 18 were sampled weekly from June-September and 3 were sampled bi-weekly from July-August. The remaining 6 growing areas were sampled as part of illness investigations or explicitly to expand the state's pulsed field gel electrophoresis (PFGE) library. Among the weekly monitoring sites, all but the Quilcene Bay site are on commercially harvested tidelands. Hood Canal 9 samples were initially collected at the DeNotta Beach site and later taken from the Belfair/Port Dock site due to an ongoing outbreak investigation during the sampling period. All other monitoring sites remained constant throughout the season. The sites were as follows (and see Figure 5):



Figure 5. Map of sampling site locations.

Weekly Monitoring Sites		Bi-Weekly Monitoring Sites	
Growing Area	Site	Growing Area	Site
Dabob Bay	Tarboo Bay	Bay Center	Goose Point harvest site
Hammersley Inlet	Orca Lane	Grays Harbor	Brady's
Hood Canal 3	Taylor's Brinnon site	Nahcotta	North of boat basin
Hood Canal 4	Beacon Point	Random Sampling Sites	
Hood Canal 5	Hamma Hamma		
Hood Canal 6	Gold Coast Union site	Growing Area	Site
Hood Canal 7	Sisters Point & Montanos	Burley Lagoon	Taylor's
Hood Canal 8	McDermott site	Hood Canal 2	Fisherman Harbor
Hood Canal 9	Denotta / Belfair Dock	Hood Canal 3	Jackson Cove
North Bay	Taylor beach in Victor	Pickering Pass	Market-ready sample
Oakland Bay	Chapman Cove	Port Gamble Bay	Port Gamble Tribe
Quilcene Bay	Yacht club launch ramp	Port Townsend	Skunk Island
Samish Bay	East (Taylor) & West (Blau)		
Skookum Inlet	Wallins		
Totten Inlet	Gallagher's Cove & Oyster Bay		



Among the 311 samples, 23 errors were reported with regard to the collection of samples and laboratory error in processing samples. Of those samples 6 were not usable; 4 were not processed due to the sample being received over temperature (10°C) and 2 were processed for surveillance purposes only. The remaining 16 samples with errors noted were all able to be processed. The following errors were noted from the 2013 *Vp* season oyster tissue collection:

Number of Samples	Type of Error	Action Taken
3	Sample received underweight	Sample processed
6	Sample received overweight	Sample processed
3	Sample received after 24 hr. time limit	Sample processed (all coastal samples)
4	Lab error	Results reported (cannot report first dilution of MPN series or range reported)
2	Temperature increased from time of collection to sample delivery	Sample processed (results for surveillance only)
4	Sample received over temperature	Sample not processed

In addition to collection and testing errors, some samples lacked complete temperature information. A total of 15 samples were missing air and tissue temperature data, 13 were missing shore temperature data, and 33 were missing surface water temperature data. Most missing temperature data resulted from thermometer and backup thermometer malfunction. Additionally, some environmental conditions (e.g., early morning September tides, extreme low tide events at Samish Bay) made it impossible, impractical, or unsafe to collect temperature data and resulted in missing data.

## **Research Studies:**

### **Pacific Shellfish Institute**

Health partnered with regional and national organizations to conduct research studies during the 2013 *Vp* season. Health partnered with the **Pacific Shellfish Institute** (PSI) to research potential *Vp* level reductions in oysters through relay, which is a process through which oysters are moved from one harvest site to another. For this study, oysters were moved from a harvest site with high levels of *Vp* to a site with low levels of *Vp*. This study was conducted in July-August where oysters were relayed in harvest bags and grow out bags from a Jackson Cove beach (in Hood Canal 3) to Skunk Island (in Port Townsend Bay). A total of 10 samples were relayed from Hood Canal 3 to Port Townsend. Each sample date included background samples from Hood Canal 3 and Port Townsend as well as relayed samples in harvest bags and in grow out bags.

The study found that the oysters relayed to Port Townsend purged *Vp* at a faster rate than environmental background samples taken at Hood Canal 3. It took 2-14 days to reduce *Vp* levels. More research is needed to better understand and validate these results. In addition, handling practices

seemed to have a strong influence on the efficacy of this practice. PSI hopes to continue the study in 2014, while expanding it to examine the differences between ground and suspended culture and to investigate further the effects of handling practices.

## Food and Drug Administration

Health partnered with the **Food and Drug Administration** (FDA) to research *Vibrio* levels before, during, and after harvest under multiple harvest scenarios. Research was conducted from July-August. The purpose of the study was to analyze the differences in *Vibrio* levels in oyster tissue following the typical harvest practices of re-submergence and wet storage. The sample design for each component of the study was as follows:

Re-submergence of harvest bags and tubs: At the Oyster Bay sampling site in Totten Inlet on the day of containerization (process of taking oysters off the beach and placing them into harvest bags or tubs), two oyster samples were taken at first exposure to air by the outgoing tide (initial exposure) and two more samples were taken just prior submergence by the incoming tide (maximum exposure). These samples provided data on the increase in *Vibrio* levels during tidal exposure. During tidal exposure on the same day, oysters were containerized into harvest bags and tubs following standard industry practices.

The containerized oysters were submerged over the tidal cycle. On the following day near high tide, two samples were collected near the center of each of two tubs. The same number of samples were collected from harvest bags to determine if there were differences in bacterial levels between harvest practices. Although the initial study design including obtaining a corresponding number of oysters harvested from the beach for determination of background *Vibrio* levels, this step was not possible as harvest occurs near high tide when the beach is fully submerged. Each sample consisted of a minimum of six oysters.

Samples were processed using standard procedures for MPN enumeration as recommended in the BAM and NSSP Model Ordinance. After overnight enrichment, MPN tubes with visible growth were tested by real-time PCR for total *Vp* and *Vv*. Each tube was also streaked to TCBS and mCPC for standard culture confirmation by DNA probe. We replicated sampling and enumeration four times, focusing on harvest dates where mid-day low tides occur. With each sample, water temperature, tissue temperature and ambient air temperature were noted.

Re-submergence to wet storage: In the Hood Canal 8 growing area the same sample collection protocols were followed as above for initial and maximum exposure samples. During tidal exposure on the same day, oysters were containerized into harvest bags following standard industry practices.

The containerized oysters were submerged over the tidal cycle. On the following day near high tide, two samples were collected from harvest bags. Although the initial study design including obtaining a corresponding number of oysters harvested from the beach for determination of background *Vibrio* levels, this step was not possible as harvest occurs near high tide when the beach is fully submerged. Each sample consisted of a minimum of six oysters.

Oysters were then transported to wet storage. Oyster samples were collected upon arrival at the wet storage facility, and again after 1, 3, 7 and 14 days from re-submerged bags and the re-circulating tanks. Some logistical challenges limited the number of samples actually taken from wet storage, but there were enough samples to compare the differences among storage types even though the data on how the levels changed by day was not available. Each sample consisted of a minimum of 6 oysters. Samples were processed using standard procedures for MPN enumeration as recommended in the BAM and NSSP Model Ordinance. After overnight enrichment, MPN tubes with visible growth were tested by real-time PCR for total *Vp* and *Vv*. Each tube was also streaked to TCBS and mCPC for standard culture confirmation by DNA probe. We replicated sampling and enumeration four times, focusing on harvest dates where mid-day low tides occur. With each sample, water temperature, tissue temperature and ambient air temperature were noted.

Despite high within-sample variation, there appeared to be a trend of *Vibrio* levels increasing with intertidal exposure and then decreasing at the time of harvest when compared to both the initial and maximum exposure samples (Appendix A). This trend was more pronounced in the Totten Inlet samples compared to the Hood Canal 8 samples, which may be related to the generally cooler water temperatures at Totten Inlet. The re-submerged bags tended to have slightly lower *Vibrio* levels than the re-submerged tubs, and both appeared to have lower levels than exposed oysters. It appears that the harvest practices of re-submergence and wet storage do not lead to a riskier product or higher *Vibrio* levels.

### National Oceanographic and Atmospheric Administration

Health partnered with the **National Oceanographic and Atmospheric Administration** (NOAA) to conduct a research study comparing *Vp* concentrations in the water column at high tide with levels in the water column at low tide and in oyster tissue samples. The experiment ran from July 16 - September 13 and consisted of collecting 10 tissue and water samples weekly for comparison with the Environmental Sample Processor (ESP) samples taken at high tide every 3 days. Samples were collected from Taylor Shellfish's facility in Samish Bay. *Vp* levels were generally very low throughout the study.

### Internal Research Studies

Health also continued a 2012 study to compare levels of *Vp* in **Pacific oysters and Kumamoto oysters** from Oakland Bay. Two years of data for this study have indicated that there may be no clear differences in *Vp* levels between the two oyster species. Both Pacific and Kumamoto samples followed a similar pattern during the season with levels rising in June and July, reaching their highest levels in late July and early August, and falling in late August (Figure 6). When just the 2013 paired samples are compared, the levels are indistinguishable between the species (Figure 7). Health does not plan to continue this comparison sampling in 2014.

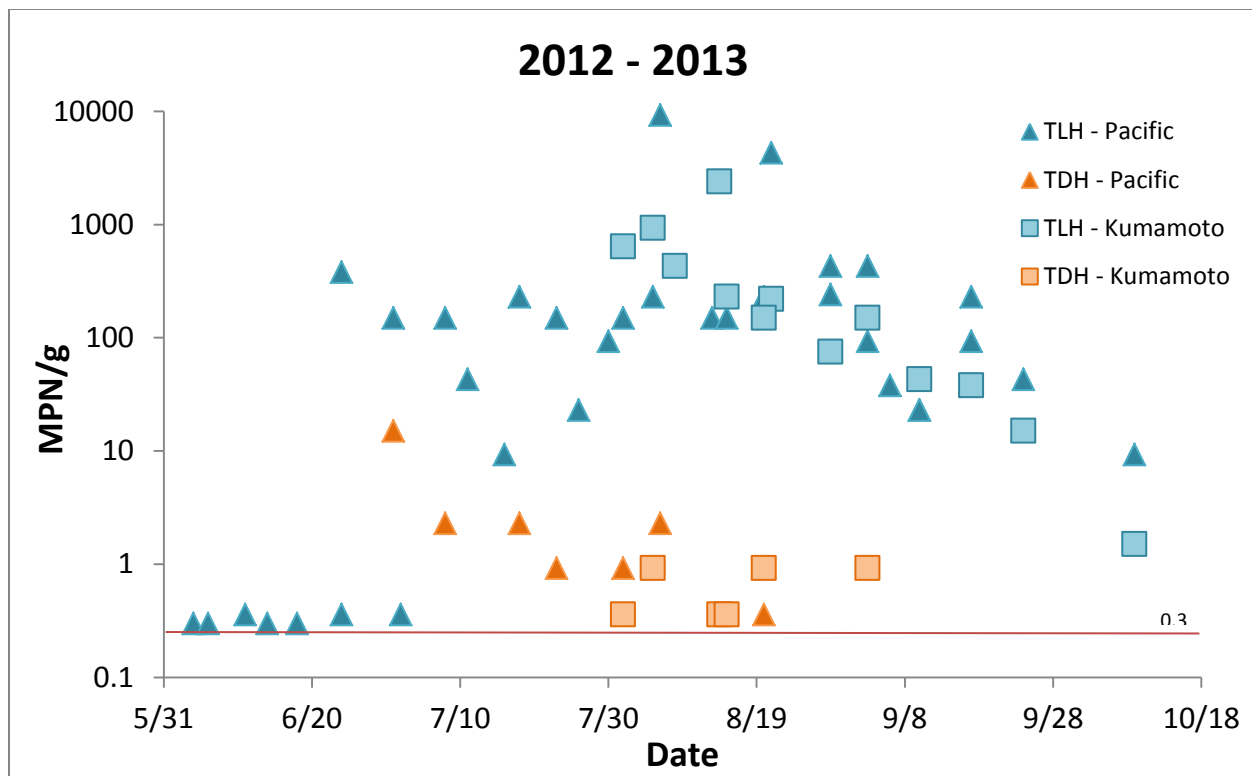


Figure 6. All 2012 and 2013 results for *Vp* levels in Pacific and Kumamoto oysters.

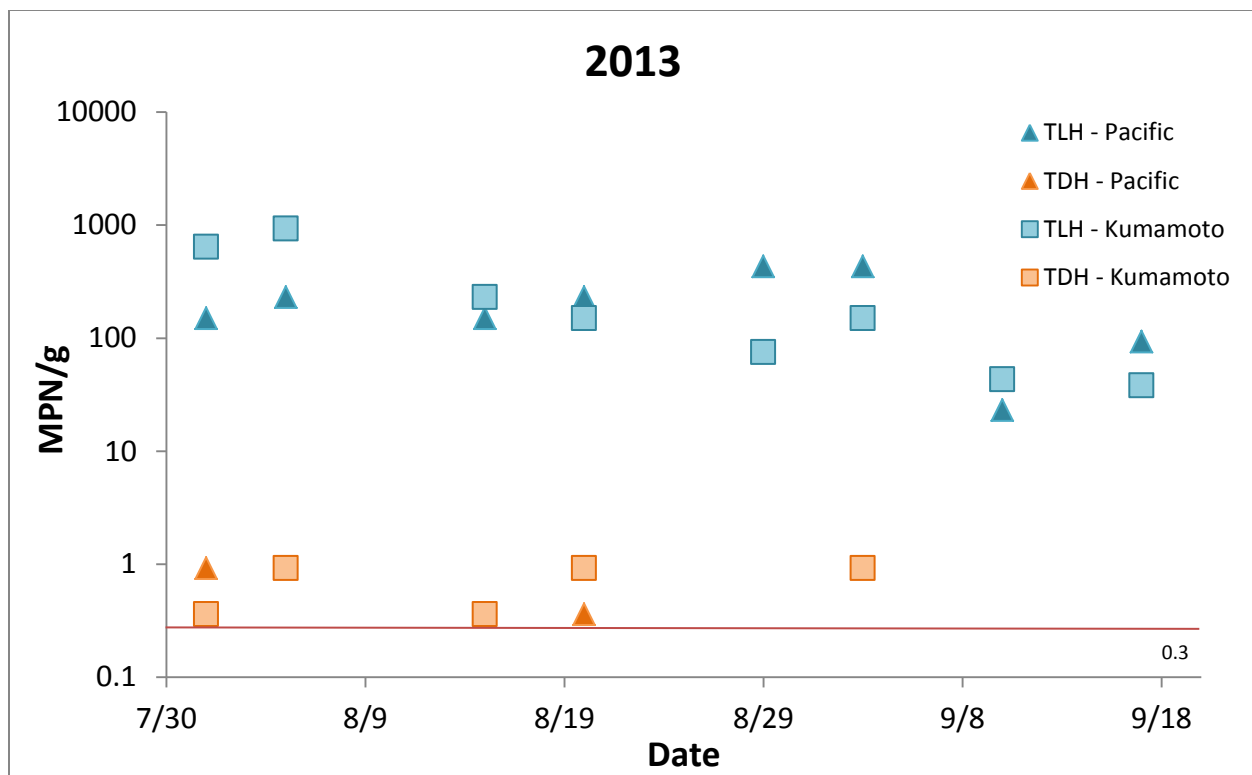


Figure 7. Subset of 2013 results only comparing paired samples of Pacific and Kumamoto oysters.

In another study, Health compared the *Vp* concentrations in **shellstock to shucked meats** from the same lot. The study specifically compared shellstock to oysters shucked and jarred that same day and oysters shucked and jarred from the same lot but kept in refrigeration for approximately one week (specific days depended on weekly sampling schedule). All samples were harvested from Hama Hama's Hood Canal 5 site. This study was a follow-up study to one conducted in 2012 that only compared freshly shucked product to shellstock from the same lot. In pooling and analyzing the 2012-2013 data, the data follows a similar pattern during the season with levels rising in June and July, reaching their highest levels in late July and early August, and falling in late August (Figure 8). When the 2013 data are analyzed by lot, there variation in the results, but there appears to be a trend where *Vp* levels decline from the initial shucked meat sample to the week old refrigerated sample (Figure 9). Interestingly, the levels in the freshly shucked product are sometimes higher than in the shellstock. Given the limited sampling data and the potential role of shucked meats in causing illness, this study may be continued in 2014.

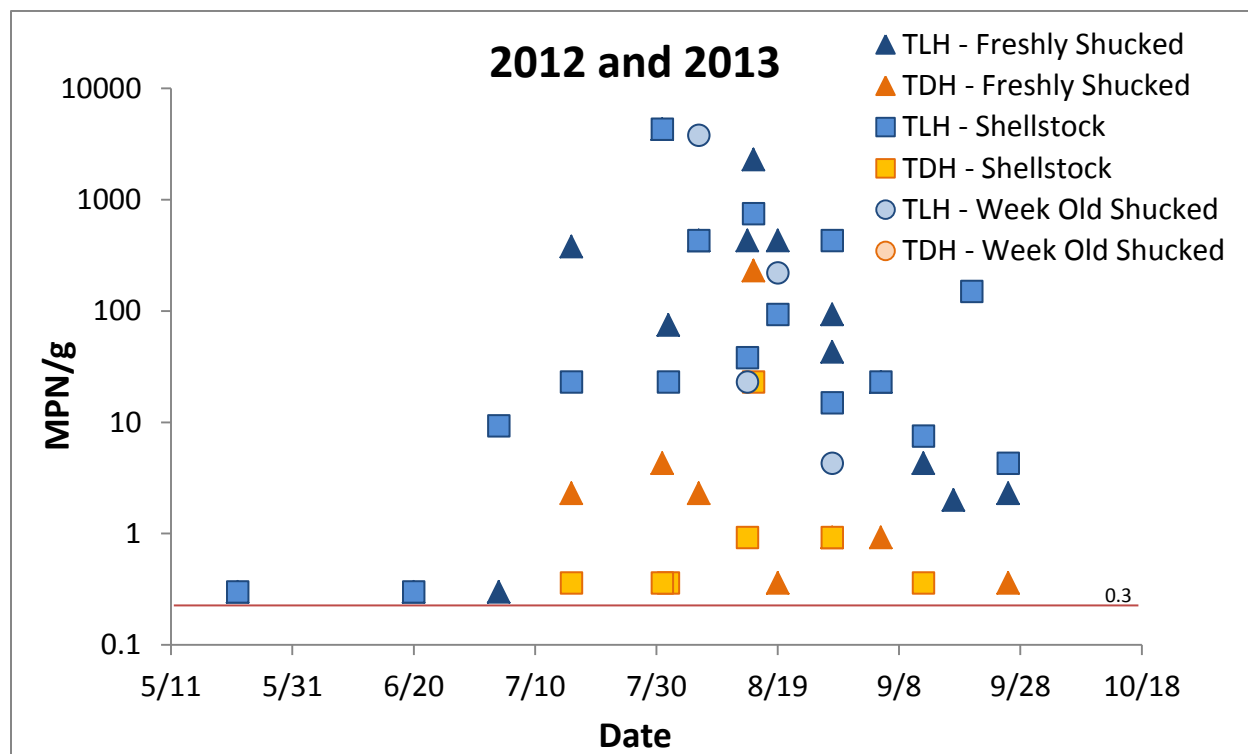


Figure 8. All 2012 and 2013 results of shellstock, freshly shucked and week old shucked meat oysters.



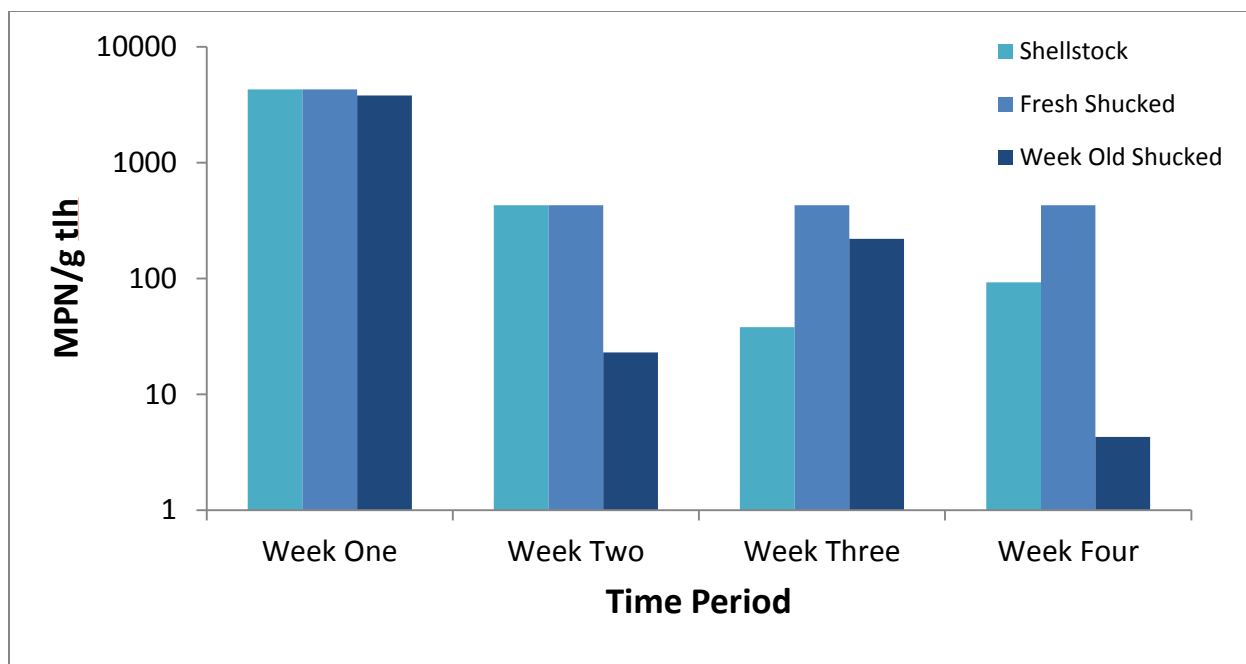


Figure 9. Comparison of result where each week represents one oyster lot of shellstock, freshly shucked and week old shucked product.

Health also conducted **comparisons of environmental conditions and Vp levels among sites within growing areas**. The comparisons for this study were:

Growing Area	Site Name
Hood Canal 7	North shore (Sisters Point)
	South shore (Montanos Beach)
Samish Bay	East (Taylor Shellfish)
	West (Blau)
Totten Inlet	Gallaghers Cove
	Oyster Bay

In addition to these comparisons, there was an opportunity to sample two sites within the Hood Canal 3 growing area due to the PSI relay study. The regular monitoring site in Hood Canal 3 is at Brinnon. When high Vp levels (above the Model Ordinance action level) were

found at the Jackson Cove site during the relay study, Health began sampling there as well. Since this was not a planned comparison and the samples were collected and submitted to the PHL by PSI, the results of this comparison should be considered with caution. The sampling followed similar protocols, but PSI's methods are somewhat different and include sampling higher on the beach. In addition, the timeframe for the sampling was much shorter than the planned comparisons at Hood Canal 7, Samish Bay, and Totten Inlet.

Overall there were slight differences in mean values between the sites with regard to temperatures and Vp levels (Figure 10-11). Although there were slight variations, the differences are not in most cases statistically significant ( $p > 0.05$ ). The differences between the Brinnon and Jackson Cove sites in Hood Canal 3 were most striking, but misleading given the low sampling effort at Jackson Cove. Sample results associated with the above mentioned internal studies can be found in Appendix B.

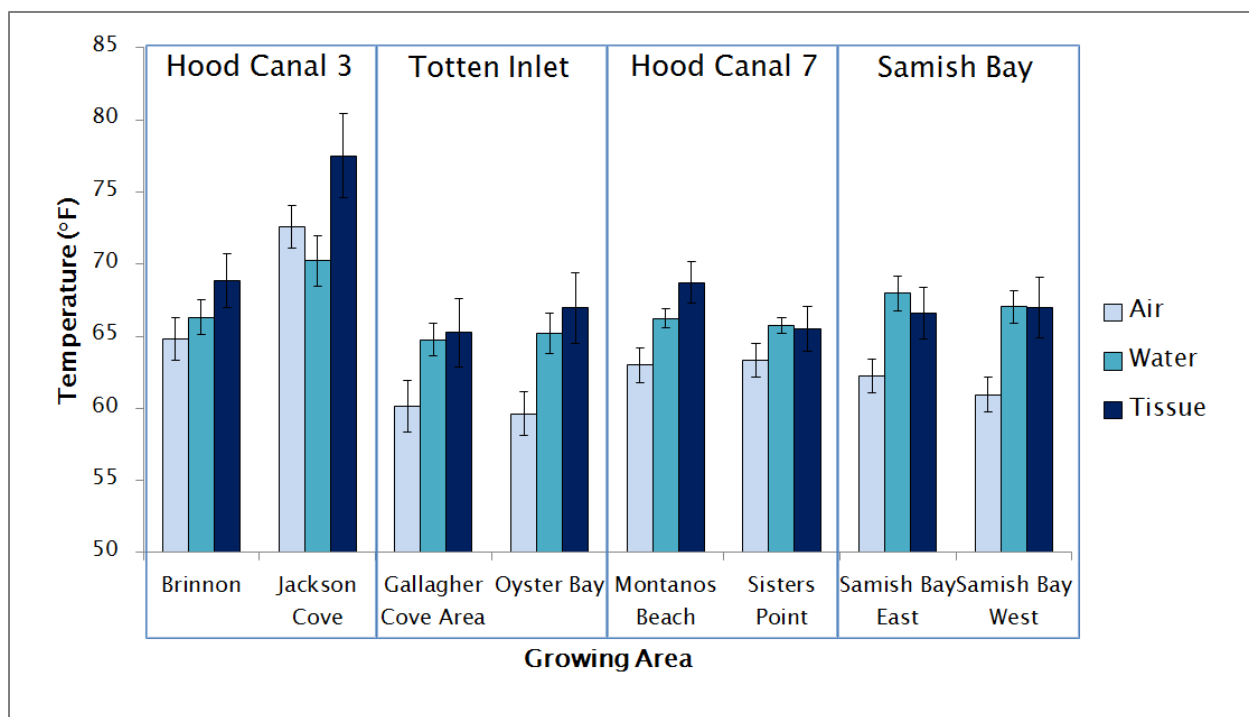


Figure 10. Variations in temperatures within and between sites in the comparison growing areas.

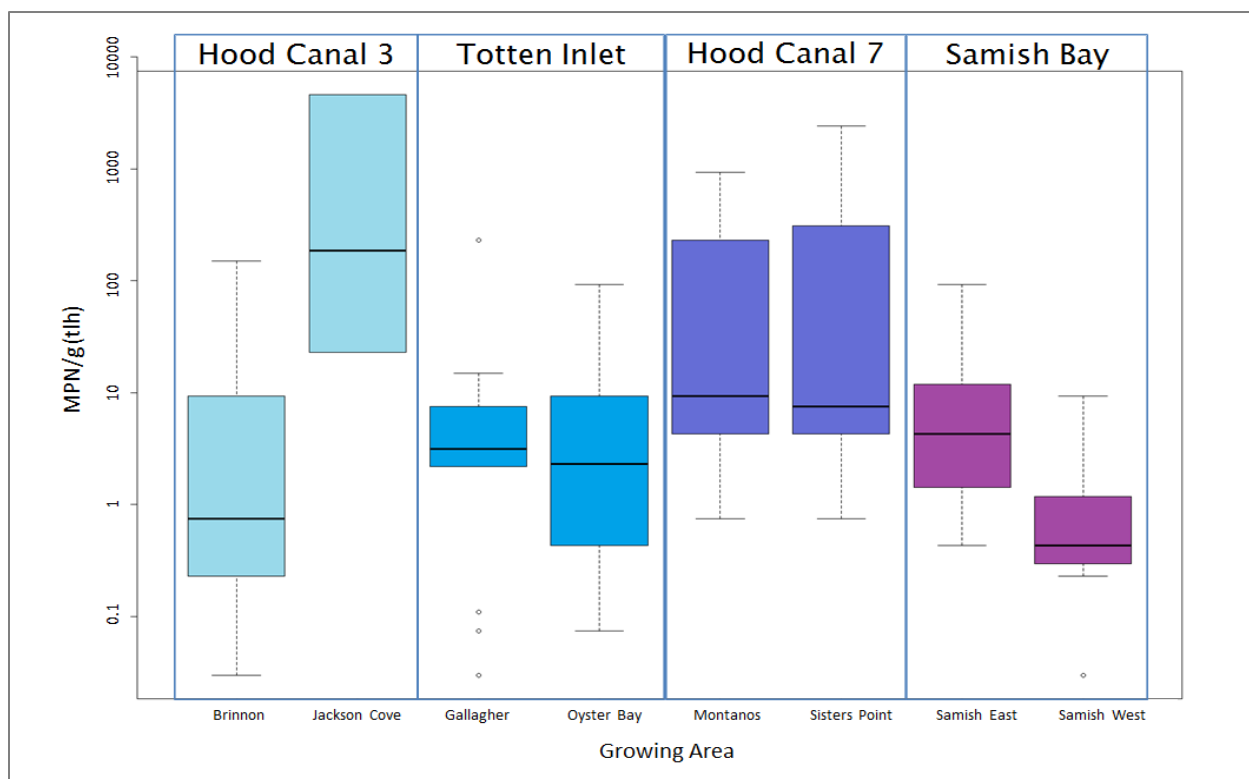


Figure 11. Box plots of Vp levels within and between sites in the comparison growing areas.

Health also collected samples from previously unsampled locations **to expand the state's PFGE library**. These samples were collected opportunistically due to illness investigations and other activities. These sites included:

Growing Area	Site Name
Burley Lagoon	Burley Lagoon
Eld Inlet	Eld Inlet
Hood Canal 2	Fisherman Harbor
Pickering Pass	Pickering Pass
Port Gamble Bay	Port Gamble Bay
Port Townsend	Skunk Island

### **Datalogger Deployment:**

OSWP collected temperature data from 18 *Vp* monitoring sites in order to better understand the relationships between temperature, *Vp* growth and illness occurrence.

In addition to these 18 sites, two additional sites not historically associated with *Vp* illness were monitored (Eld

Inlet and Port Townsend Bay). The results of this study were combined with other data and are being used to inform a proactive *Vp* management approach for the State of Washington. See the Risk Based Management of Marine Pathogens and Biotoxins: *Vibrio* Project Final Report (PC-00J32601-4) and Quality Assurance Project Plan for additional information regarding the deployment of temperature data loggers and how the data is being used by OSWP.

## Appendix A

FDA results associated with the 2013 re-submergence study.

Totten Inlet Study	Trial 1				Trial 2				Trial 3				Trial 4			
	Vv	tlh	tdh	trh	Vv	tlh	tdh	trh	Vv	tlh	tdh	trh	Vv	tlh	tdh	trh
Initial exposure		NA			930	430	0.72	43	62	1500	3	15	150	7.2	0.15	1.5
		NA			930	430	2.1	4.3	43	150	20	75	0.15	43	0.15	0.3
Maximum exposure	300	15000	2.1	4300	930	1500	15	93	15000	4300	0.72	430	0.15	1500	300	610
	1500	24000	1.4	1500	430	2100	4.3	24	27	4300	27	430	0.15	930	11	20
Resubmerged bags	1.1	240	0.36	7.5	2	9.3	0.15	0.36	0.15	93	0.15	0.3	1.1	43	1.5	3.8
	280	9300	0.36	2.3	4.3	24	0.15	0.92	0.15	15	0.61	0.15	0.61	150	1.1	0.3
	21	24	0.92	2.3	0.3	4.3	0.15	0.92	15	240	0.36	0.92	0.72	9300	240	240
	11	93	0.36	2.3	2	9.3	0.36	0.36	0.15	9.3	0.36	2.3	30	24000	1500	930
Resubmerged tubs	29	93	0.36	9.3	0.15	11	0.36	1.5	0.15	150	3.6	0.92	280	9300	240	240
	6.1	29	0.72	2.8	12	24	0.15	0.74	1.5	240	0.3	0.92	110	24000	75	43
	7.2	43	0.92	2.3	2.7	75	0.36	0.15	0.15	240	0.3	0.74	1600	1200	4.3	4.3
	0.74	93	0.15	7.5	93	16	0.36	0.92	0.15	430	0.15	2.1	6.2	930	11	21

Hood Canal 8 Study	Trial 1				Trial 2				Trial 3			
	Vv	tlh	tdh	trh	Vv	tlh	tdh	trh	Vv	tlh	tdh	trh
Initial exposure	24	9300	<0.3	150	30	3800	0.3	9.3	0.3	1500	380	150
	4.3	9300	<0.3	210	11	210	<0.3	2.3	3	24000	240	240
Maximum exposure	12	2400	<0.3	9.3	72	7500	<0.3	7.5	0.61	9300	<0.3	9.3
	12	7500	<0.3	15	61	2400	<0.3	43	11	4300	<0.3	12
Harvest	14	930	0.36	24	1100	7500	<0.3	43	0.72	2400	<0.3	1.1
	15	9300	<0.3	21	<0.3	210	0.92	7.5	1.1	430	<0.3	0.36

Note: Site located at A&K Shellfish harvest site, not the weekly Vp monitoring site (McDermott).

Penn Cove Study	Raft				Tank			
	<i>Vv</i>	<i>tlh</i>	<i>tdh</i>	<i>trh</i>	<i>Vv</i>	<i>tlh</i>	<i>tdh</i>	<i>trh</i>
7/25/2013	140	750	0.36	1.5	7.2	7500	0.74	9.3
7/27/2013	0.92	240	0.15	0.36	29	4300	240	240
7/31/2013	93	430	0.15	0.36	0.92	7500	0.15	4.3
8/4/2013	6.2	430	0.15	0.36	0.15	240	0.15	0.92
8/7/2013	0.15	120	11	7.2	0.15	43	11	20
8/12/2013	1.1	430	0.15	0.15	360	240	0.15	0.15
8/26/2013	61	4300	0.15	0.15	210	4300	0.15	0.36
8/29/2013	280	24000	0.36	0.36	0.15	43000	0.15	0.15



## Appendix B

Washington State Department of Health Environmental results associated with 2013 research studies. The complete set of *Vp* monitoring data for 2013 can be accessed at: S:\EPH\SF\LICENSE & CERTIFICATION SECTION\Vibrio Program\Charts and Data or accessed through the Shellfish Sample System.

Note that although the reporting units were standardized in the main text and figures (converted to MPN/1.0g), *tdh* is reported in MPN/0.1g by the PHL. The units in Appendix B have not been converted, *tlh* is reported as MPN/1.0g and *tdh* is reported as MPN/0.1g.

Samish Bay Tissue Sample Results (NOAA study):

Vibrio#	Date & Time Collected	Low Tide	Air Temp (°F)	Surface Water Temp (°F)	Shore Water Temp (°F)	Tissue Temp (°F)	TLH MPN/ 1.0g	TDH MPN/0.1g
201300099	7/17/2013 8:45	06:30AM	69.08	72.68	72.50	73.04	150	<0.030
201300129	7/24/2013 12:24	12:10PM	60.08		69.98	59.00	93	<0.030
201300144	7/31/2013 8:35	07:00AM	54.50		65.66	58.46	930	0.036
201300171	8/6/2013 14:40	11:00AM	62.78		81.32	68.18	240	0.036
201300192	8/14/2013 7:03	04:50AM	53.96		60.08	60.44	43	<0.030
201300230	8/21/2013 12:30	11:10AM	63.14		77.00	72.32	22	<0.030
201300243	8/28/2013 7:02	04:50AM	59.18	65.48	62.42	65.12	64	<0.030
201300268	9/4/2013 11:15	10:30AM	61.52	67.10	67.46	65.66	43	<0.030
201300287	9/11/2013 6:30	03:09AM				50.00	9.3	<0.030
201300292	9/16/2013 9:00	08:22AM	60.08	64.40	63.14	63.68	9.3	<0.030

Comparison of Pacific Oysters and Kumamoto Oysters:

Vibrio#	Date & Time Collected	Low Tide	Air Temp (°F)	Surface Water Temp (°F)	Shore Water Temp (°F)	Tissue Temp (°F)	TLH MPN/1.0g	TDH MPN/0.1g	Notes
201300158	8/1/2013 9:44	10:00AM	58.64	66.92	67.1	65.3	640	0.036	Kumamoto
201300156	8/1/2013 9:45	10:00AM	58.64	66.92	67.1	63.68	150	0.092	Pacific
201300162	8/5/2013 12:03	12:50PM	64.94	70.16	71.96	87.08	930	0.092	Kumamoto
201300164	8/5/2013 12:05	12:50PM	64.94	70.16	71.96	84.74	230	<0.03	Pacific
201300204	8/15/2013 8:50	08:00AM	63.14	65.66	67.64	67.1	230	0.036	Kumamoto
201300205	8/15/2013 8:50	08:00AM	63.14	65.66	67.64	67.46	150	<0.03	Pacific
201300221	8/20/2013 13:00	01:00PM	63.32	72.5	72.86	84.2	150	0.092	Kumamoto
201300223	8/20/2013 13:00	01:00PM	63.32	72.5	72.86	80.24	230	0.036	Pacific
201300251	8/29/2013 7:45	08:10AM	65.66	67.46	66.38	66.02	75	<0.03	Kumamoto
201300248	8/29/2013 7:45	08:10AM	65.66		66.38	66.38	430	<0.03	Pacific
201300263	9/3/2013 12:25	12:28PM	63.14	68	69.26	72.5	150	0.092	Kumamoto
201300261	9/3/2013 12:25	12:28PM	63.14	68	69.26	75.02	430	<0.03	Pacific
201300285	9/10/2013 0:00	03:45AM					43	<0.03	Kumamoto
201300286	9/10/2013 0:00	03:45AM					23	<0.03	Pacific
201300298	9/17/2013 12:30	11:53AM	62.06	67.64	71.06	71.06	38	0.03	Kumamoto
201300299	9/17/2013 12:30	11:53AM	62.06	67.64	71.06	72.68	93	<0.03	Pacific

Comparison of Shellstock and Shucked Meat:

Vibrio#	Date Collected	Low Tide	Air Temp (°F)	Surface Water Temp (°F)	Shore Water Temp (°F)	Tissue Temp (°F)	TLH MPN /1.0g	TDH MPN/ 0.1g	Notes
201300146	7/31/13 12:00 AM	05:45AM					4300	0.43	Freshly shucked meat
201300148	7/31/13 6:39 AM	05:45AM	53.96	57.92	57.02	57.02	4300	0.036	Shellstock
201300170	8/6/13 12:00 AM	11:25AM					430	0.23	Freshly shucked meat
201300174	8/6/13 10:05 AM	11:25AM	63.68	68.18	69.44	67.64	430	<0.03	Shellstock
201300169	8/6/13 12:00 AM	11:25AM					3800	0.036	Week old shucked meat
201300198	8/14/13 12:00 AM	05:10AM					430	0.03	Freshly shucked meat
201300194	8/14/13 6:38 AM	05:10AM	56.3	64.94	58.28	55.58	38	0.092	Shellstock
201300197	8/14/13 12:00 AM	05:10AM					23	<0.03	Week old shucked meat
201300209	8/19/13 12:00 AM	10:10AM					430	0.036	Freshly shucked meat
201300211	8/19/13 8:25 AM	10:10AM	62.6	64.58	65.12	64.04	93	0.03	Shellstock
201300210	8/19/13 12:00 AM	10:10AM					220	<0.03	Week old shucked meat
201300247	8/28/13 12:00 AM	05:08AM					43	<0.03	Freshly shucked meat
201300242	8/28/13 6:45 AM	05:08AM	61.52	62.6	60.98	63.14	15	<0.03	Shellstock
201300246	8/28/13 12:00 AM	05:08AM					4.3	<0.03	Week old shucked meat
201300270	9/4/13 12:00 AM	11:05AM						<0.03	Freshly shucked meat
201300273	9/4/13 9:12 AM	11:05AM	63.86	62.42	62.78	62.78	9.3	<0.03	Shellstock
201300269	9/4/13 12:00 AM	11:05AM						<0.03	Week old shucked meat

Hood Canal 7 Comparison:

Vibrio#	Date & Time Collected	Low Tide	Site Name	Air Temp (°F)	Surface Water Temp (°F)	Shore Water Temp (°F)	Tissue Temp (°F)	TLH MPN/1.0g	TDH MPN/0.1g
201300001	6/4/2013 10:44	09:30AM	Sisters Point	72.5	65.12	68	71.96		
201300003	6/4/2013 11:30	09:30AM	Montanos Beach	72.7	69.8	80.24	80.06	23	<0.030
201300024	6/12/2013 12:50	02:02PM	Sisters Point	58.8		63.86	63.86	75	<0.030
201300021	6/12/2013 14:21	02:00PM	Montanos Beach	68	64.58	64.76	69.98	75	<0.030
201300029	6/19/2013 7:45	08:22AM	Montanos Beach	56.1	60.8	62.24	60.26	93	<0.030
201300051	6/26/2013 13:22	12:52PM	Sisters Point					43	<0.030
201300048	6/26/2013 13:27	01:52PM	Montanos Beach	67.6	68	67.82	77	930	0.23
201300059	7/1/2013 7:18	07:10AM	Montanos Beach	70.2	70.88	68.9	69.26	9300	<0.300
201300058	7/1/2013 7:27	07:10AM	Sisters Point	64	64.94	68	64.94	430	0.074
201300077	7/8/2013 10:49	12:53PM	Sisters Point	59	64.94	64.94	62.96	24000	0.03
201300073	7/8/2013 11:11	11:53AM	Montanos Beach	63.1	68.72	68.9	72.14	3800	0.036
201300107	7/18/2013 6:43	07:50AM	Sisters Point	56.1	66.02	64.94	58.1	1500	<0.030
201300111	7/18/2013 7:45	07:50AM	Montanos Beach	57.4	66.2	66.38	59	2300	<0.030
201300126	7/23/2013 11:16	12:05PM	Montanos Beach	55.9	60.98	60.08	66.92	9300	0.036
201300121	7/23/2013 11:16	12:05PM	Sisters Point	66.9	68	69.08	82.04	4300	<0.030
201300151	8/1/2013 6:50	08:00AM	Sisters Point	54	60.98	60.08	55.94	4300	<0.030
201300155	8/1/2013 8:15	08:00AM	Montanos Beach	57.2	65.3	64.94	59.54	430	<0.030
201300161	8/5/2013 10:35	11:00AM	Montanos Beach	63.9	66.38	67.1	73.94	4300	<0.030
201300166	8/5/2013 10:48	11:00AM	Sisters Point	64	64.94	64.04	71.96		<0.030
201300202	8/15/2013 5:56	06:15AM	Sisters Point	64	65.84	64.94	64.22	2200	<0.030
201300206	8/15/2013 7:32	06:15AM	Montanos Beach	63.5	66.02	65.3	64.94	150	<0.030
201300218	8/20/2013 10:20	11:00AM	Sisters Point	68	67.1	66.92	68.36	75	<0.030
201300225	8/20/2013 11:30	11:00AM	Montanos Beach	60.1	67.1	67.28	72.68	43	<0.030
201300249	8/29/2013 6:30	06:20AM	Montanos Beach	65.3	66.92	66.38	66.74	43	<0.030
201300255	8/29/2013 7:15	06:20AM	Sisters Point	65.1	68	63.86	65.3	43	<0.030

201300256	9/3/2013 9:54	10:30AM	Sisters Point	63.3	66.02	65.48	64.4		
201300259	9/3/2013 11:01	10:30AM	Montanos Beach	60.8	64.94	64.76	68.72	9.3	<0.030
201300290	9/11/2013 4:43	03:42AM	Sisters Point	59.9		65.3	60.26	23	<0.030
201300300	9/17/2013 10:55		Montanos Beach	63.9	65.48	66.2	67.82	7.5	<0.030
201300304	9/18/2013 11:00	09:49AM	Sisters Point	64.4	63.5	63.5	60.62	7.5	<0.030



Samish Bay Comparison:

Vibrio#	Date & Time Collected	Low Tide	Site Name	Air Temp (°F)	Surface Water Temp (°F)	Shore Water Temp (°F)	Tissue Temp (°F)	TLH MPN/1.0g	TDH MPN/0.1g
201300032	6/19/2013 10:00	08:30AM	Samish Bay - East	62.6	69.44	71.78	67.82	4.3	<0.030
201300031	6/19/2013 11:15	08:30AM	Samish Bay - West	66.38	71.24	72.32	73.76	<0.300	<0.030
201300042	6/25/2013 12:25	12:15PM	Samish Bay - West	64.94	65.3	67.82	70.7	<0.300	<0.030
201300043	6/25/2013 13:45	12:28PM	Samish Bay - East	68.54		78.44	72.86	4.3	<0.030
201300066	7/2/2013 8:02	07:48AM	Samish Bay - West	64.04	73.4	71.24	68.18	3.8	<0.030
201300065	7/2/2013 8:40	07:48AM	Samish Bay - East	65.3	71.24	71.78	72.14	38	0.15
201300079	7/9/2013 11:01	11:56AM	Samish Bay - West	67.64	74.84	79.16	84.2	38	<0.030
201300078	7/9/2013 11:45	11:56AM	Samish Bay - East	69.44		88.34	79.34	150	0.23
201300098	7/17/2013 8:00	06:30AM	Samish Bay - West	66.2	68.54	72.86	68.9	9.3	<0.030
201300099	7/17/2013 8:45	06:30AM	Samish Bay - East	69.08	72.68	72.5	73.04	150	<0.030
201300130	7/24/2013 11:28	12:10PM	Samish Bay - West	51.08	59	60.98	66.02	4.3	<0.030
201300129	7/24/2013 12:24	12:10PM	Samish Bay - East	60.08		69.98	59	93	<0.030
201300143	7/31/2013 7:55	07:00AM	Samish Bay - West	55.04	67.46	66.38	57.92	15	<0.030
201300144	7/31/2013 8:35	07:00AM	Samish Bay - East	54.5		65.66	58.46	930	0.036
201300172	8/6/2013 11:45	11:00AM	Samish Bay - West	64.04	71.42	70.7	72.14	93	0.092
201300171	8/6/2013 14:40	11:00AM	Samish Bay - East	62.78		81.32	68.18	240	0.036
201300193	8/14/2013 6:00	04:50AM	Samish Bay - West	55.76	67.64	66.92	54.86	4.3	0.074
201300192	8/14/2013 7:03	04:50AM	Samish Bay - East	53.96		60.08	60.44	43	<0.030
201300229	8/21/2013 11:30	11:10AM	Samish Bay - West	59.54	66.74	67.28	74.48	4.3	<0.030
201300230	8/21/2013 12:30	11:10AM	Samish Bay - East	63.14		77	72.32	22	<0.030
201300244	8/28/2013 6:30	04:50AM	Samish Bay - West	59.36	64.22	62.42	58.28	2.3	<0.030
201300243	8/28/2013 7:02	04:50AM	Samish Bay - East	59.18	65.48	62.42	65.12	64	<0.030
201300267	9/4/2013 10:18	10:30AM	Samish Bay - West	61.52	63.14	61.88	62.6	15	0.074
201300268	9/4/2013 11:15	10:30AM	Samish Bay - East	61.52	67.1	67.46	65.66	43	<0.030
201300287	9/11/2013 6:30	03:09AM	Samish Bay - East				50	9.3	<0.030

201300291	9/16/2013 8:30	08:22AM	Samish Bay - West	59.9	61.88	59.54	60.08	4.3	0.036
201300292	9/16/2013 9:00	08:22AM	Samish Bay - East	60.08	64.4	63.14	63.68	9.3	<0.030

Totten Inlet Comparison:

Vibrio#	Date & Time Collected	Low Tide	Site Name	Air Temp (°F)	Surface Water Temp (°F)	Shore Water Temp (°F)	Tissue Temp (°F)	TLH MPN/1.0g	TDH MPN/0.1g
201300006	6/6/2013 9:45	11:30AM	Gallagher Cove Area	68.72	63.86	66.02	71.78	<0.300	<0.030
201300005	6/6/2013 10:40	11:30AM	Oyster Bay	69.8	65.48	69.62	76.64	4.3	0.092
201300019	6/11/2013 13:35	01:30PM	Gallagher Cove Area	65.66	66.74	71.6	71.24	0.74	<0.030
201300016	6/11/2013 14:24	01:30PM	Oyster Bay	59.54	68.36	72.5	73.22	0.92	<0.030
201300039	6/24/2013 12:57	01:15PM	Gallagher Cove Area	64.76	67.1	69.08	72.86	1.1	<0.030
201300038	6/24/2013 13:49	01:15PM	Oyster Bay	66.2	71.42	71.06	75.74	4.3	<0.030
201300062	7/1/2013 7:00	08:01AM	Gallagher Cove Area	62.96	60.08	60.98	60.98	22	0.93
201300063	7/1/2013 7:45	08:01AM	Oyster Bay	60.98	60.08	60.08	61.52	12	<0.030
201300091	7/10/2013 12:35	01:57PM	Gallagher Cove Area	66.2	72.14	75.02	78.98	93	0.036
201300088	7/10/2013 13:45	01:57PM	Oyster Bay	67.46	76.46	77.9	82.76	930	0.23
201300095	7/16/2013 6:20	06:50AM	Oyster Bay	52.88	63.32	61.16	56.3	150	0.074
201300096	7/16/2013 7:35	06:50AM	Gallagher Cove Area	54.68	65.84	61.88	59.36	43	0.036
201300114	7/22/2013 11:25	12:10PM	Gallagher Cove Area	53.96	60.08	62.06	60.98	23	<0.030
201300115	7/22/2013 12:11	12:10PM	Oyster Bay	55.94	62.06	62.96	68	15	<0.030
201300139	7/30/2013 7:37	07:10AM	Gallagher Cove Area	48.92	63.68	55.04	51.08	150	0.23
201300138	7/30/2013 8:15	07:10AM	Oyster Bay	48.92	53.06	51.98	50	93	9.3
201300179	8/7/2013 12:08	01:00PM	Oyster Bay	63.5	66.74	69.08	76.1	430	0.38
201300178	8/7/2013 12:48	01:00PM	Gallagher Cove Area	70.16	71.24	73.76	80.6	2300	0.75
201300190	8/13/2013 5:50	05:10AM	Gallagher Cove Area	52.88	64.04	63.5	53.06		
201300189	8/13/2013 6:20	05:10AM	Oyster Bay	53.42	63.14	61.34	54.86		
201300215	8/19/2013 11:03	11:05AM	Gallagher Cove Area	53.96	55.94	60.98	64.94	23	<0.030
201300216	8/19/2013 11:38	11:05AM	Oyster Bay	55.04	60.98	62.06	66.02	43	<0.030
201300236	8/27/2013 6:15	05:12AM	Gallagher Cove Area	59	66.2	62.06	59.9	75	0.036
201300238	8/27/2013 6:40	05:12AM	Oyster Bay	58.28	65.84	62.78	60.08	23	<0.030
201300278	9/5/2013 10:50	12:37PM	Gallagher Cove Area	61.16	63.5	63.5	67.64	43	<0.030

201300277	9/5/2013 11:38	12:37AM	Oyster Bay	61.34	65.3	66.2	67.82	9.3	<0.030
201300279	9/10/2013 0:00	02:47AM	Oyster Bay					23	<0.030
201300280	9/10/2013 4:00	02:47AM	Gallagher Cove Area					23	0.036
201300307	9/18/2013 13:06	11:50AM	Oyster Bay	62.96	69.98	71.96	75.02	3.8	<0.030
201300309	9/24/2013 0:00	03:35AM	Oyster Bay					0.74	0.036

Hood Canal 3 Comparison:

<b>Vibrio#</b>	<b>Date &amp; Time Collected</b>	<b>Low Tide</b>	<b>Site Name</b>	<b>Air Temp (°F)</b>	<b>Surface Water Temp (°F)</b>	<b>Shore Water Temp (°F)</b>	<b>Tissue Temp (°F)</b>	<b>TLH MPN/1.0g</b>	<b>TDH MPN/0.1g</b>
201300145	7/31/2013 0:00		Jackson Cove					15000	0.23
201300150	7/31/2013 8:36	07:00AM	Brinnon	55.94	64.04	53.96	60.08	230	0.036
201300173	8/6/2013 12:02	11:24AM	Brinnon	70.88	69.26	71.24	79.88	220	<0.030
201300182	8/7/2013 12:20	12:00PM	Jackson Cove	71.24	75.56	75.56	87.8	46000	0.75
201300187	8/12/2013 14:30		Jackson Cove	77	72.5	72.5	79.88	46000	0.43
201300195	8/14/2013 5:02	05:05AM	Brinnon	56.66	68.9	57.02	60.26	43	0.036
201300217	8/19/2013 10:10		Jackson Cove	68	69.98	69.98	75.56	230	0.092
201300208	8/19/2013 10:30	10:02AM	Brinnon	68.54	68.54	64.4	72.32	93	<0.030
201300241	8/28/2013 0:00	05:07AM	Jackson Cove					230	<0.030
201300240	8/28/2013 6:05	05:14AM	Brinnon	61.52	58.64	58.46	60.98	7.5	<0.030



